In the Description

Please amend the paragraph commencing at page 21, line 17, as follows.

Figure 6 shows a protein sequence alignment of the members of the triacylglycerol lipase gene family (SEQ ID Nos: 13-15). Shaded residues are identical to the LLGXL protein (SEQ ID NO: 8). The deduced amino acid sequence of human LIPG(EL; endothelial lipase) (SEQ ID NO: 8) is provided on the top line and is compared with the other major members of the TG lipase family, LPL (lipoprotein lipase) (SEQ ID NO: 13), HL (heptaic lipase) (SEQ ID NO: 14) and PL (pancreatic lipase) (SEQ ID NO: 15). EL residues identical to those in at least one other member of the family are shaded as well as the corresponding residue in the other family member. Amino acids are numbered according to convention beginning with the initial residue of the secreted protein. The predicted sites of signal peptide cleavage are marked with a solid line between amino acid residues. The GXSXG lipase motif containing the active serine is boxed. The amino acids of the catalytic triad are marked with an asterisk. The conserved cysteines are marked with filled circles. Potential N-linked glycosylation sites are marked with arrowheads. The lid region is indicated by a bold line. Gaps were introduced into the sequences to maximize the alignment values using the CLUSTAL program.

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Please amend the paragraph commencing at page 22, line 23, as follows.

Figure 10 shows the sequence of the immunizing peptide (SEQ ID NO: 16) and its relation to the LLGXL protein sequence (SEQ ID NO: 8). The peptide is shown in the shaded box. The terminal cysteine was introduced to aid coupling of the peptide to the carrier protein.

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Please amend the paragraph commencing at page 23, line 13, as follows.

G3 Figure 13 shows the sequence of the rabbit LIPG PCR product (RLLG.SEQ, SEQ ID NO: 12) and the sequence alignment between the rabbit LIPG PCR product and the corresponding sequence in the human cDNA (LLG7742A) (SEQ ID NO: 7). Identical nucleotides are shaded.

Please insert the following five paragraphs at page 25, between lines 7 and 8.

The present invention relates to a polypeptide encoded by the LIPG gene and any analogue, fragment, derivative, or mutant thereof which is derived from such polypeptide and which retains at least one biological property thereof (hereafter, collectively, "LIPG polypeptide", "LIPG protein", "endothelial lipase" or "EL", or "LLG"). The LIPG polypeptide is a member of the triacyglycerol lipase family which includes also hepatic lipase (HL), lipoprotein lipase (LPL), and pancreatic lipase (PL). Unique to LIPG polypeptide in comparison with the other members of the triacylglycerol lipase family is that it has been found to be synthesized by endothelial cells.

G4 The present invention relates also to compositions and methods for regulating the levels of HDL cholesterol and apolipoprotein AI, VLDL cholesterol and LDL cholesterol. The compositions and methods of the present invention function by raising or lowering the levels of LIPG polypeptide.

LIPG polypeptide has the ability to lower the levels of HDL cholesterol and apolipoprotein AI as well as the levels of VLDL cholesterol and LDL cholesterol. In addition, LIPG is abundantly expressed in the placenta and a role for this enzyme in development is possible, given the importance of lipid transport in fetal development

(Farese et al., *Trends Genet.*, 14: 115-120 (1998)). Given HDL's beneficial properties including the reduction of the risk of atherosclerotic cardiovascular disease, it is desirable to raise HDL levels in a patient by lowering the enzymatic activity of LIPG polypeptide. Given the correlation of high levels of LDL and VLDL and increased risk of atherosclerotic diseases, it is desirable to lower the level of these compounds in a patient afflicted with high levels thereof by raising the enzymatic activity of LIPG polypeptide.

The sequence of LIPG polypeptide includes several aforementioned features which are common to the members of the triacyglycerol lipase family: (A) a GXSXG motif; (B) a "catalytic triad"; (C) a heparin-binding region; and (D) a "lid region". As stated previously, the lid region forms an amphipathic helix covering the catalytic pocket of the enzyme (Winkler et al., *Nature*, 343: 771-774 (1990); van Tilbeurgh et al., *J. Biol. Chem.*, 269: 4626-4633 (1994)) and confers substrate specificity to the enzymes of the triacylglycerol lipase family (Dugi et al., *J. Biol. Chem.*, 270: 25396-25401 (1995)). The lid region diverges significantly between members of the triacylglycerol lipase family. The lid region of LIPG polypeptide consists of 19 residues and is three residues shorter and less amphipathic than those found in LPL and HL, which is consistent with the fact that LIPG polypeptide has a different enzymatic profile from LPL and HL. The sequence of the lid region of LIPG polypeptide is shown in Figure 6 (the sequence between two framing cysteine residues in the sequence indicated by a bold line). LIPG polypeptide comprises also an approximately 39 kD catalytic domain of the triacylglycerol lipase family, e.g., having the sequence SEQ ID NO: 10.

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There exist two major forms of LIPG polypeptide: LLGXL polypeptide and LLGN polypeptide, either of which may exist in glycosylated or non-glycosylated form. Human LLGXL polypeptide (which includes SEQ ID NO.: 8) has 500 amino

acids and a molecular weight of approximately 55 kD. The human LLGXL polypeptide exhibits 43% similarity to human lipoprotein lipase and 37% similarity to human hepatic lipase. Human LLGN polypeptide (which includes SEQ ID NO.: 6) has 354 amino acids and a molecular weight of approximately 40 kD. It is believed that a 68 kD form of LIPG polypeptide is likely a glycosylated form of human LLGXL polypeptide. SEQ ID NO.: 6 contains the same first 345 amino terminal residues as does SEQ ID NO.: 8. This common sequence is SEQ ID NO.: 10. In addition to this common sequence, SEQ ID NO.: 6 contains an additional nine unique residues while SEQ ID NO.: 8 contains an additional 146 unique residues.--

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Please delete the paragraphs commencing at page 25, line 9, and at page 26, line 6.

Please amend the paragraph commencing at page 96, line 10, as follows.

A commercially available lambda cDNA library derived from rabbit lung tissue (Clontech, Cat. #TL1010b) was used to isolate a fragment of the rabbit homolog of the LIPG gene. Five microliters of the stock library were added to 45 ml water and heated to 95°C for 10 minutes. The following were added in a final volume of 100 ml: 200 mM dNTPs, 20 mM Tris-HCl pH 8.4, 50 mM KCl, 1.5 mM $MgCl_2$, 100 mM each primer DLIP774 and LLGgen2a, and 2.5 U Taq polymerase (GIBCO). The reaction was thermocycled 35 times with the parameters of: 15 seconds at 94°C, 20 seconds at 50°C and 30 seconds at 72°C. Ten microliters of the reaction were analyzed via agarose gel electrophoresis. A product of approximately 300 basepairs was detected. A portion (4 ml) of the reaction mix was used to clone the product via the TA cloning system. The insert of a resulting clone was sequenced (SEQ ID NO: 11). An alignment between the deduced rabbit amino acid sequence (SEQ ID NO: 12) and the corresponding sequence of the human cDNA is also shown in Figure 13. Of the

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nucleotides not part of either amplification primer, there is an 85.8% identity between the rabbit and human LLG sequences. The predicted protein encoded by this rabbit cDNA shares a 94.6 % identity with that of the human protein, with most of the nucleotide substitutions in the third or "wobble" positions of the codons. Notably, this region spans the "lid" sequence of the predicted LLG proteins and is a variable domain in the lipase gene family. This is evidence that there is a high degree of conservation of this gene between species.

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Please amend the paragraph commencing at page 103, line 25, as follows.

To perform the experiments discussed in Examples 12 to 16, the following procedure (based on the procedure outlined in Example 1) was used to obtain the cDNA for LIPG. THP-1 cells were plated in the presence of phorbol 12-myristate 13-acetate (PMA, 40 ng/ml; Sigma) for 48 hours. The differentiated THP-1 cells were exposed for 24 hours to either oxLDL (50 μg/ml) or control medium. Total RNAs were collected and purified using standard procedures. Poly(A)$^+$ RNA was purified from total RNA using a poly-dT magnetic bead system (Promega). cDNA synthesis and PCR amplification were accomplished using protocols from the Differential Display kit, version 1.0 (Display Systems Biotechnology). The primer pairs that yielded the initial cDNA fragment of EL were downstream primer 7 (5'-TTTTTTTTTTTGA-3') (SEQ ID NO: 17) and upstream primer 15 (5'-GATCCAATCGC-3') (SEQ ID NO: 18). The amplification reaction was fractionated on a 6% nondenaturing acrylamide sequencing format gel and an amplification product found only in the reaction containing cDNA from THP-1 cells exposed to oxLDL was identified and excised from the gel. A reamplification using the same primers was performed and the product was excised and subcloned into the pCRII vector using the TA cloning system (Invitrogen). Insert sizes were determined using *EcoR*I digestions of the plasmids, and clones containing inserts of the approximate

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size of the original PCR product were sequenced using fluorescent dye-terminator reagents (Prism, Applied Biosystems) and an Applied Biosystems 373 DNA sequencer. We extended the cDNA sequence of the original, gel-excised cDNA using the 5'-RACE system (GIBCO). RNA (1 μg) from the THP-1 cells used initially in the differential display reactions was used in the 5'-RACE procedure using a gene-specific primer (5'-TAGGACATGCACAGTGTAATCTG-3') (SEQ ID NO: 19) for first strand cDNA synthesis. We performed PCR amplification of the cDNA using an anchor primer and gene-specific primer 2 (5'-GATTGTGCTGGCCACTTCTC-3') (SEQ ID NO: 20). This reaction (1 μl) was used in a nested re-amplification using the universal amplification primer (5'-CUACUACUACUAGGCCACGCGTCGACTAGTAC-3') (SEQ ID NO: 22) and the gene-specific primer 3 (5'-GACACTCCAGGGACTGAAG-3') (SEQ ID NO: 21) to increase levels of specific product for subsequent isolation. The reaction products were cloned into the pCRII vector from the TA cloning kit and the sequence determined. A human placental cDNA library (oligo dT and random primed) was obtained from Clontech and probed with the 5'-RACE reaction PCR product. The DNA from hybridizing clones was purified using LambdaSorb reagent (Promega). Inserts were excised from the phage DNA by digestion with *EcoR*I, subcloned into the *EcoR*I site of the Bluescript II SK plasmid vector (Stratagene), and sequenced.

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Please amend the paragraph commencing at page 105, line 17, as follows.

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A 17-residue peptide (GPEGRLEDKLHKPKATC) (SEQ ID NO: 16) was synthesized corresponding to residues 8-23 of the secreted LIPG gene product on a Model 433A peptide synthesizer (Applied Biosystems). Peptide (2 mg) was coupled to maleimide-activated keyhole limpet haemocyanin (2 mg) following the protocols included in the Imject Activated Immunogen Conjugation kit (Pierce Chemical). After desalting, one-half of the conjugate was emulsified with an equal volume of

Freund's complete adjuvant (Pierce) and injected into a New Zealand White rabbit. Four weeks after the initial inoculation, a booster inoculation was administered with an emulsification made exactly as described above except for the use of Freund's incomplete adjuvant (Pierce). Two weeks after the boost, the titres of specific antibodies were determined in a test bleed via ELISA using immobilized peptide.

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Please amend the paragraph commencing at page 106, line 6, as follows.

HUVECs were propagated in a commercially prepared endothelial cell growth medium (EGM, Clonetics) supplemented with bovine brain extract (3 mg/ml; Clonetics), whereas HCAECs were propagated in EGM with bovine grain extract (3 mg/ml) and 5% fetal bovine serum. Cultures were stimulated by addition of PMA (100 ng/ml). After 24 hours incubation, RNA was extracted from the cells via the Trizol method, electrophoresed on a 1% agarose-formaldehyde gel, transferred to Nytran membrane on a Turboblotter apparatus (Schleicher and Schuell) and crosslinked to the membrane using a Stratalinker ultraviolet crosslinker (Stratagene). The 5'-RACE reaction PCR product was radiolabelled using the random priming technique. The radiolabelled probe (1-2x10^6 cpm/ml) was denatured by heating to 95 °C for 10 minutes and quick-chilled on ice before adding to the filter in QuikHyb. Hybridization was allowed to proceed for 3 hours at 65 °C. Filters were exposed to Kodak XAR-2 film with intensifying screens at -80 °C. We incubated HUVEC- and HCEAC-conditioned medium with heparin-Sepharose CL-6B at 4 °C for 1 hour. After centrifugation, the pelleted heparin-Sepharose was suspended in SDS loading buffer, heated to 95 °C for 5 minutes and loaded onto a 10% Tris-Glycine SDS gel (NOVEX). After electrophoresis at 140 V for 90 minutes, the proteins were transferred to nitrocellulose membranes and detected with rabbit anti-LIPG peptide antisera (1:5,000), with goat anti-rabbit peroxidase conjugated antisera (1:5,000; Boehringer) as the secondary antibody. The membranes were reacted with

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